PATENT

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Applicant : WISNIEWSKI et al. Group Art Unit: 3743

Serial No.: 08/895,936 Examiner : John Ford

Filed : July 17, 1997

For : FREEZING AND THAWING VESSEL WITH THERMAL BRIDGE FORMED BETWEEN HEAT EXCHANGE MEMBERS

Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF RICHARD WISNIEWSKI

1. I am one of the inventors of the above-referenced United States patent application. I am also a named inventor of six U.S. Patents relating to cryopreservation of biopharmaceuticals and numerous pending patent applications. I make the statements herein to the best of my own personal knowledge.

2. I received degrees in Mechanical Engineering and Chemical Engineering from Warsaw Technical University in Warsaw, Poland in 1971. I have over 26 years of experience in applied research, process and product development, process control, equipment and device design, industrial facility design and project and team management in the biopharmaceutical field.

3. I am a co-founder and currently the Chief Technology Officer of Integrated Biosystems, Inc.

4. Prior to my current position, I have held senior engineering and management positions with Wyeth-Ayerst, Genentech, Inc., Bepex Corporation and Ares Serono. While at

Genentech, Inc., I was a Principal Process Engineer responsible for pioneering work in the design of equipment and processes for biopharmaceutical manufacturing, including systems for cryopreservation, chromatography, filtration and bioreactors and aseptic processing used in large scale production.

5. I have published numerous articles in the areas of cryobiology and cryopreservation. While I was working for Genentech, Inc., I co-published, with Vincent L. Wu, an article entitled "Large-Scale Freezing and Thawing of Biopharmaceutical Drug Product" for the Advanced Technologies For Manufacturing Of Aseptic & Terminally Sterilized Pharmaceuticals & Biopharmaceuticals convention during the Proceedings of the International Congress in 1992 ("the 1992 article"). I have provided a copy of this article in Exhibit A. This 1992 article is similar to the 1996 article previously disclosed to the Patent Office during the prosecution of the above-reference application.

6. The 1992 article discloses a freeze-thaw vessel for biopharmaceutical products having an internal heat transfer coil with fins welded to the external surface of the coil pipe which I designed. The figure on page 134 of the article accurately depicts the heat transfer coil and fin arrangement within the vessel. This article does not disclose or suggest the formation of a thermal transfer bridge, as defined in the above-reference application, by the medium in a gap between the fins and the interior wall of the vessel, even after the medium in the gap is frozen.

7. In Exhibit B, I have provided a schematic representation of the freezing which would have occurred in the vessel disclosed in the 1992 article at a period in time before the medium between the fin and the interior wall of the vessel is frozen along with a graph showing the temperature distribution along the radius of the vessel. Exhibit B depicts a schematic sectional view of the interior of the vessel disclosed in the 1992 article. To the best of my knowledge the temperature graph reasonably resembles the temperature profile along the line (R-R) at different points. For example, at the center of the pipe within the fin the temperature is Tc, while the temperature at the edge of the fin is Tft. As shown in

this graph, the temperature in the gap between the fin and the interior wall increases and then decreases from the distal end of the fin to the interior wall. This temperature distribution occurs because the gap between the distal end of the fin and interior wall is too large. Accordingly, no thermal bridge is formed because heat is not transferred from the fin through the medium in the gap to the interior wall. Rather, heat is transferred from a location in the gap between the fin and the interior wall to both the fin and the interior wall.

8. In Exhibit C, I have provided a similar schematic view of the same vessel. However, the freezing is at a period in time when the frozen medium built up on the fin meets the frozen medium built up on the interior wall. To the best of my knowledge the temperature graph reasonably resembles the temperature distribution along the line (R-R) at different points. As shown in this graph, the temperature in the gap between the fin and the interior wall increases and then decreases from the distal end of the fin to the interior wall. This temperature distribution occurs because the gap between the distal end of the fin and interior wall is too large. Accordingly, no thermal bridge is formed because heat is not transferred from the fin through the medium in the gap to the interior wall. Rather, heat is transferred from a point in the gap between the fin and the interior wall to both the fin and the interior wall.

9. In Exhibit D, I have provided a similar schematic view of the same vessel. However, the freezing is at a period in time when the medium in the gap between the fin and the interior wall is completely frozen. To the best of my knowledge the temperature graph reasonably resembles the temperature distribution along the line (R-R) at different points. As shown in this graph, the temperature in the gap between the fin and the interior wall increases and then decreases from the distal end of the fin to the interior wall, even when the medium is frozen. This temperature distribution occurs because the gap between the distal end of the fin and interior wall is too large. Accordingly, no thermal bridge is formed, even after the medium in the gap is frozen, because heat is not transferred from the fin through the medium in the gap to the interior wall. Furthermore, even after

additional freezing may occur, including total freezing within the vessel, no thermal bridge is formed in the gap area. Rather, heat is transferred from a point in the gap between the fin and the interior wall to both the fin and the interior wall.

10. With respect to the above-referenced application, a thermal bridge will not form if the gap between the heat transfer members and the interior wall of the vessel is too large, even after the medium in the gap is frozen. If this gap is too large, heat would be transferred from a location within the gap to both the heat transfer member and the interior wall similar to the Genentech device, not from the heat transfer member to the interior wall as required by the formation of a thermal bridge.

11. I declare under penalty of perjury under the laws of the United States of America that the foregoing information contained in this Affidavit is true and correct.

January _23__, 2002 _____
Richard Wisniewski

Proceedings of the International Congress

Advanced Technologies For Manufacturing Of Aseptic & Terminally Sterilized Pharmaceuticals & Biopharmaceuticals

Basel, Switzerland
17-19 February 1992
Convention Center Basel

Presented By The Parenteral Drug Association, Inc.
In Cooperation With The Association Pour Les Produits Parenteraux et Steriles (A3P) And The Parenteral Society

Advanced Technologies for Manufacturing of
Aseptic and Terminally Sterilized Pharmaceuticals
and Biopharmaceuticals

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Convention Center Basel
Basel, Switzerland

BIOPHARMACEUTICALS

CLOSURE TECHNOLOGY

FILLING

AUTOMATION

LARGE-SCALE FREEZING AND THAWING OF BIOPHARMACEUTICAL DRUG PRODUCT

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ABSTRACT

This study reports the successful implementation of mechanical-based refrigeration systems and vessels designed for the freezing and thawing of bulk protein solutions. Design principles for large- scale, freeze-thaw refrigeration equipment, freeze-thaw vessel, and controls are discussed. Freezing and thawing process validation methods and the effects on protein product are presented. The purified bulk product can be sterile filtered into the freeze-thaw vessel, frozen, and stored in a stable state until product is needed for filling. The frozen drug product may be shipped, thawed, pooled, filtered, and ultimately filled into vials. The stainless steel freeze-thaw tank design is compatible with current pharmaceutical manufacturing operations including product transfer, clean- in-place, and steam-in-place procedures. The authors believe that large-scale freezing is a viable and economical alternative for the intermediate storage and handling of sensitive bulk biopharmaceutical drug product.

Key Words

Convection	Freeze-thaw
Cryoconcentration	Heat Transfer
Eutectic	Protein Stability
Formulation	System Design

Introduction

Depending on the stability and storage period desired biological products are often stored at 2-8 °C, -20 °C, -70 °C or are lyophilized. The ability to freeze the formulated bulk protein product in a portable stainless steel vessel sized to the batch yield has many advantages. Since the yields of biotechnology products are relatively small and the difference in cost and time for filling and finishing small versus large production lots is minimal, the ability to store and pool offers an economy of scale advantage. Per unit cost quality control expense is also reduced. The ability to store bulk material for prolonged periods of time allows for campaigning of production facilities while maintaining the flow of product. The ability to store sensitive product in the frozen state allows transport of product that may not otherwise withstand liquid shipment. Product with short liquid shelf-life that is sensitive to protease degradation or deamidation may be frozen in bulk volumes prior to lyophilization.

The effect of long-term freezer storage, thawing and refreezing of select proteins in serum have been investigated by DiMagno, et al (1). Many of the hormones reported were stable at -20 °C and -70 °C for up to ten years. Schiewe and Rau reviewed processes and available equipment for deep freezing in biological and medical applications (2).

Upon the outset of the project there was no satisfactory method available to freeze and thaw large volumes of protein solutions under the conditions complying with the cGMPs. Freezing in small containers such as blood bags or vials is labor intensive requiring filling and capping equipment in addition to the freeze-thaw systems and agitation equipment for thawing. Product transfer, storage, recovery and handling in a portable stainless steel vessel were deemed superior to alternative methods.

Freezing

The freezing process involves solidification phenomenon with the solid-liquid interface moving and latent heat release at the interface. The latent

heat has to be removed by heat conduction through the solidified layer of material and through the heat exchange surfaces.

The movement of a solid-liquid interface may cause a phenomenon of redistribution of solutes. If the concentration of the solutes at the interface exceeds the diffusional effects for solute molecules, then cryoconcentration occurs. This phenomenon of solute redistribution has been analyzed by many researchers (3,4,5).

Korber and Scheiwe have provided experimental evidence confirming this phenomenon as well as an analysis of dendritic growth or branching ice crystal growth (6,7). Granger et. al., have developed computational methods to analyze the solute redistribution by moving solid-liquid interface and related effects (3).

For large volumes of freezing solutions, such as the system described here, solute redistribution effects may be of lesser significance due to natural convection effects and due to the relatively low velocity of the moving solid-liquid interface. It is predicted that natural convection plays a more significant role in larger volumes of liquid due to larger temperature gradients within the bulk. The low velocity of the liquid-solid interface in the large-scale system may allow greater solute diffusional effects reducing the effect of solute redistribution at the solid-liquid front.

If the growth of dendrites is comparable to the diffusional rate of dissolved molecules, for example proteins in this case, then the cryoconcentration phenomenon could be minimized and solutes may be entrapped between the progressing dendrites at relatively low concentrations. Smaller molecules, however, such as buffer salts in this case, may diffuse at a faster pace and be pushed in front of the dendrites with a gradually increasing concentration in the liquid phase and become excluded from the frozen mass of material. This phenomenon was examined in the designed freeze-thaw tank system and explains why samples taken at the unfrozen cavity significantly increased in ionic strength (indicating increased concentration of salts) and yet the concentration of protein at the unfrozen cavity did not differ greatly from the starting protein concentration.

There is evidence in the literature that proteins can be affected by changes occuring in the liquid phase during freezing, like increases in salt concentration and changes in pH (8,9,10,11). It was feared that during freezing, cryoconcentration effects may cause crystallization of buffer components leading to pH change which would effect protein stability or cause the protein to unfold or amino acid chains to be cleaved. Also, cryoconcentration effects combined with low temperature effects may cause a decrease in protein solubility and hence precipitation. In the system studied, the ability of the protein to maintain a certain percentage of single chain molecule and the efficacy of the drug was tested before and after repeated freeze-thaw cycles (see Figure 4).

Protein drug formulations may include a wide variety of compounds. Stabilizing agents or cryoprotective agents including for example sugars, glycols, glycerol, sodium glutamate, sodium acetate, potassium phosphate, serine and alanine may be considered during the formulation development (12,13,14). Consideration should be given to the final formulation to avoid low temperature freezing and to allow stability at warmer temperatures. For example, avoid using high concentrations of salts which may depress the freezing point, promote precipitation, or cause protein denaturation.

There is limited information available in the literature on the behavior of proteins at low temperatures (8,10,15,16,17,18,19) on formulations composition (9,20,21) and on specific cryoprotectant-type formulations (13,22,23,24), although those sources can be used rather as references only, and a need for an individual approach to each molecule of interest may be anticipated.

Protein stability and conformation can be affected by low temperature alone, for example without any significant changes occuring in solution like salt concentration or change in pH (8,16,17,18, 19,22, 24,25).

Thawing

The thawing process has quite different thermodynamic requirements when compared to freezing. During thawing, the liquid phase appears first at the heat transfer surface and it quickly separates the frozen product mass from the heating surface. Since the protein solution cannot be over-heated, the temperature of the heating surfaces should be maintained below a certain limit at which the product is stable, for example, about room temperature. At limited temperature gradients, natural convection in the liquid phase is limited even if the configuration of the heat transfer surfaces provides favorable conditions for natural convection. Research

done on natural convection during melting has shown that the influence of natural convection on acceleration of the melting rate increases with the height of the heat transfer surface (26,27). The top of a liquid cavity becomes much wider than the bottom part due to warm currents which rise along the heated surface and descend along the frozen mass boundary.

Our interest turned to methods to increase the movement within the liquid phase. An agitator in the tank was considered impractical, since the agitator would be enclosed in the frozen mass for too long to be effective. Recirculation of the thawed liquid from the bottom of the tank to the top of the frozen mass at a slow rate was found to be suitable and aseptic and allowed recirculation to occur relatively early in the thawing cycle.

To determine the point during the thawing cycle when recirculation should begin, there must be adequate liquid phase at the tank bottom and sufficient melting at the tank walls so that the liquid can be pumped from the tank bottom, to the top of the frozen mass, around the ice mass, and return to the bottom. The use of sterilized silicone tubing and a peristaltic pump was found to be suitable and aseptic for product recirculation during thawing. A product recirculation rate at two times the average melting rate was effective for thawing and for producing homogeneous bulk product by the end of the thawing process. The recirculation tubing was also useful for sampling and for transferring the product to another vessel at the end of the thawing process. See Figure 1.

Another option for providing agitation during thawing is to shake or move the entire tank on a mechanical shaker platform. In this case, frozen masses float in the liquid phase and causes stirring of the liquid phase by relative solid-liquid movement. This method is quite simple and aseptic, however it requires heavy equipment and vibrators and is more expensive to scale up.

Freeze-thaw Vessel Design

Since the product of interest was a parenteral drug, containment was a critical issue, and since the product could be subjected to a slow freezing rate, a jacketed tank system with recirculating heat transfer medium was chosen over freezing with liquid nitrogen. There are many problems with immersion of a large container in liquid nitrogen including thermal stress to the container, efficient removal of bubbling cold gas, and in general complicated handling of the container would be involved.

The problem with freezing and thawing in a jacketed tank is that heat transfer decreases significantly after reaching a certain thickness of frozen material, due to poor heat conduction through the frozen mass. Two conditions which improve heat transfer include the use of a large temperature difference between the cooling medium and the liquid being frozen, and the use of extended heat transfer surfaces.

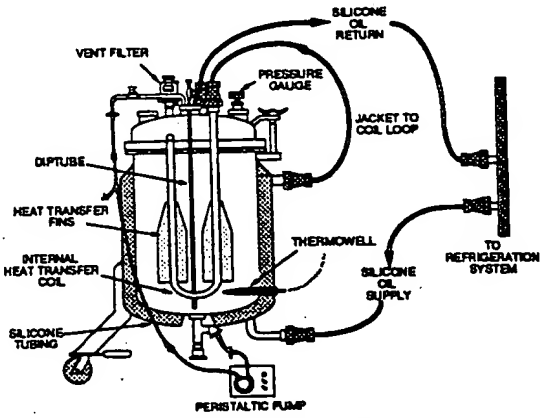

Figure 1. Freeze-thaw Vessel: Thawing Configuration

The freeze-thaw tank design could be in the form of a cylindrical, jacketed vessel of small diameter and large height. However, such a design is not economical and there is a possibility of developing significant mechanical stress in the side walls from expansion during freezing. To increase the product volume and to use a tank of more conventional proportions a heat transfer coil was added to provide additional heat transfer surface. The coil was in the form of a 3.35 meter (11 foot), one inch Schedule 40 (316L) seamless pipe with three 180 degree bends. Heat transfer fins were welded to the external surface of the pipe (28,29). The fin design was analyzed using a computer model. The fin's length, thickness and shape were designed to maintain efficient heat transfer during freezing and thawing. The fin performance characteristics are based on it having a higher thermal conductivity than the frozen material. One important requirement is that the fins must be of sufficient thickness to allow adequate heat transfer by conduction towards the wall of the pipe. The heat transfer surface configuration was designed to minimize internal mechanical stress caused by expansion of the freezing mass and to induce freezing from the bottom of the vessel upwards by providing more heat transfer surface at the tank bottom. Natural convection and surface freezing effects were taken into consideration in the fin design to prevent rapid

Several design considerations may be implemented to allow the refrigeration system to operate at the lowest possible temperatures. The backpressure valve after the evaporator can be oversized and kept almost open or it could be completely removed from the circuit if the lines are properly sized. The suction accumulator performance becomes critical especially at the end of the freezing cycle and it should be heated by electrical coils or hot gas refrigerant coils.

The condenser should be cooled with refrigerated water or glycol to keep the temperature of the condensed refrigerant as low as possible. To aid in the fine tuning of the refrigeration system, thermocouples may be attached to the refrigerant piping at critical points in the system to monitor the refrigeration system performance. These temperture readings can become a part of an overall system control and diagnostic scheme.

Another factor to consider is the properties of the heat transfer medium for freezing and thawing. It should be non-toxic, have a very low freezing point and low viscosity at low temperatures. Its thermal properties should assure sufficient heat transfer coefficients in the tank jacket and in the evaporator of the refrigeration system. During the thawing process the heat transfer medium is heated and therefore it should not evaporate or degrade during heating. It should also be non-flammable, non-corrosive and compatible with stainless steel (chloride-free).

Our attention focused on silicone fluids of low viscosity. For example, Dow 200 Fluid with 5 centistoke viscosity can achieve approximately minus 60 °C, while its boiling point is still high enough for heating during thawing. If a lower viscosity fluid is considered, such as 1 centistoke Dow 200 Fluid, then its boiling point temperature may not be well suited for the heating duty during thawing. Also low viscosity fluids have relatively low flashpoint temperatures and may pose a flammability danger in open systems.

The recommended upper operating temperature limits for the 1 and 5 centistoke Dow 200 Fluids are 32 °C and 65 °C respectively. Since the 5 centistoke fluid had a flashpoint of 133 °C it was chosen as a heat transfer medium for applications involving thawing at moderate temperatures. The viscosity of the 5 centistoke fluid at minus 60 °C is approximately 75 centistokes.

The pump used for recirculating the heat transfer medium should be designed so that the addition of heat is minimized. This can be accomplished by selecting a pump design configuration that allows effective dissipation of heat from the motor, and by implementing a pump with high pumping efficiency, working at its optimum point on the pump curve. The optimum working point on the pump curve should be determined at the higher fluid viscosity at the lower end of the working temperature range. A seal-less, leakproof canned motor pump can be suitable.

The design of the refrigerant evaporator should ensure turbulent, high velocity flow of heat transfer medium around the bundle of evaporator tubes. Moisture in the heat tranfer medium can create operational problems including ice build-up on the evaporator tubes. A moisture trap should be installed in the system such as a dessicant type filter drier cores. A multiple moisture trap design is recommended to allow an undisturbed system performance in the event that traps need to be changed during the operation.

The heater used to heat the heat transfer medium during the thawing process can be on a separate branch of the piping or it can be installed in the heat transfer fluid reservoir. An electric heating element, controlled by a proportional controller is suitable. To maintain temperature control over the heat transfer fluid during thawing a separate cooler is needed to prevent overheating of the heat transfer fluid. This cooler should be situated downstream of the heater. The cooler can be a heat exchanger with refrigerated glycol as a cooling medium, or a small mechanical refrigeration unit.

Figure 2 shows a freeze-thaw system design capable of freezing and thawing multiple tanks. The R-502 refrigeration system is utilized for freezing and the R-12 refrigeration system used in conjunction with the heater in the reservoir is utilized for thawing. Compressed air is used to recover the heat exchange media from the tank jacket and heat transfer coil at the end of the freeze and thaw processes.

General System Performance

During the freezing process, the thermal load on the cooling system varies due to an increase in the thickness of the layer of frozen material at the heat transfer surfaces. At the beginning, the heat load is high due to cooling of the tank and the liquid product and due to a release of latent heat from the first, thin layer of rapidly freezing product on the heat transfer surfaces.

freezing of the upper levels of the liquid and to avoid liquid phase entrapment under frozen surfaces.

The approach was to design the freezing vessel in such a way to minimize cryoconcentration effects and provide a uniform freezing rate with the protein molecules being occluded by the moving freezing front. The heat transfer fins were configured to divide the tank volume into compartments to decrease the freezing and thawing time and to reduce cryoconcentration effects. Compartmentation of the tank is especially effective for maintaining liquid in a static state to minimize cryoconcentration. A full external dimpled-type jacket which extended to the tank bottom was also provided and the vessel was well insulated with compressed chloride-free insulation. To improve radiant heat transfer effects and to aid cleaning, the internal surfaces of the vessel including the internal heat exchanger surfaces were polished to 320 grit (10 ra) and electropolished to provide a mirror finish.

The vessel was designed to be compatible with current pharmaceutical manufacturing operations including sterilization-in-place and clean-in-place. The vessel was constructed according to ASME code to withstand steam sterilization and was rated for full vacuum. Multiple spray devices inserted into the vessel were designed for the vessel to provide thorough cleaning of the internal heat exchange surfaces.

Refrigeration System

The refrigeration system was developed using engineering principles similar to those of a freeze-drier. It was known from laboratory experiments, that the product was able to be frozen to -20 °C and thawed at 2-8 °C in vials without deleterious effects.

Mechanical refrigeration systems used to cool the heat transfer media have limits regarding the lowest achievable temperatures, depending on the refrigerant used and the design principle. For instance, refrigerant R-502 allows temperatures in the evaporator to reach a level as low as minus 50-53 °C and the refrigerant R13B1 achieves about minus 65-67 °C with a two-stage compressor (30). Figure 3 shows the temperature of the coolant over time without a load. The versatile system which may be utilized for freezing and thawing of protein solutions should have the capability of providing well controlled, varying cooling and warming rates. The cooling rate can be controlled, for example, by oversizing the compressor and utilizing a bypass loop to the heat exchanger to control the amount of recycling of the recirculating heat exchange media. If very low temperature applications are required a cascade refrigeration design may be considered, or the heat transfer fluid may be cooled using liquefied gases.

Since the load on the refrigeration system varies significantly and there is a prolonged period of operation at a very low load at the end of the freezing cycle, selection of the evaporator expansion valve is of particular importance in avoiding evaporator flooding at low loads.

A synthetic lubricating oil for the compressor which performs well for prolonged work and at low temperature should be selected over hydrocarbon oils which break down and become viscous at low temperatures.

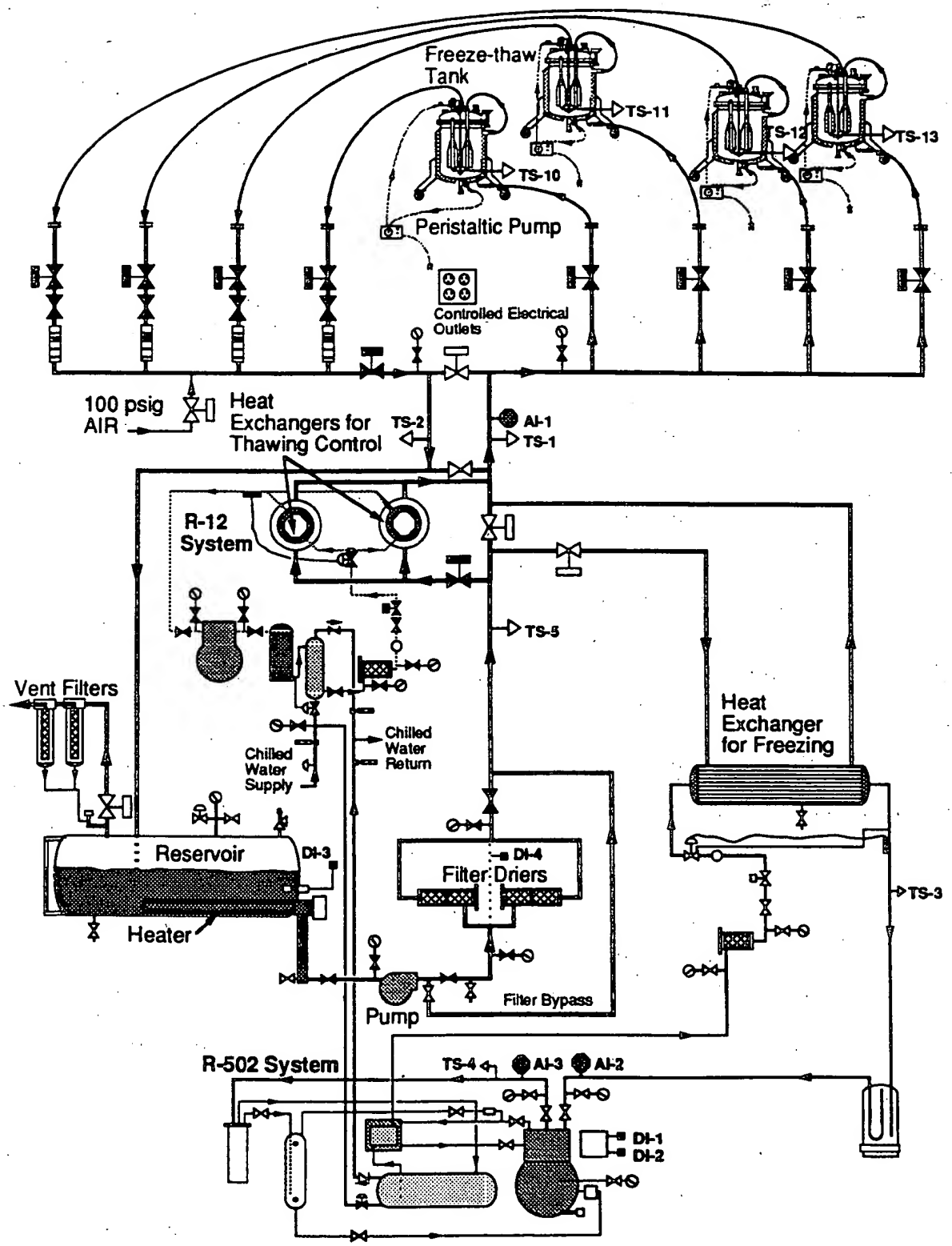

Figure 2. Refrigeration System and Freeze-thaw vessels in multiple thawing configuration

As soon as the layer of frozen material appears on the heat transfer surface, the heat flux begins to decline due to heat conduction through the frozen material and an increased resistance to heat transfer. Heat conduction through frozen material quickly becomes the controlling factor over freezing rate and overall heat transfer. Computer models have been developed to predict the heat flux decline and the movement of a freezing front. Figure 3 shows the freeze-thaw temperature profile for freezing and thawing during one of the test runs.

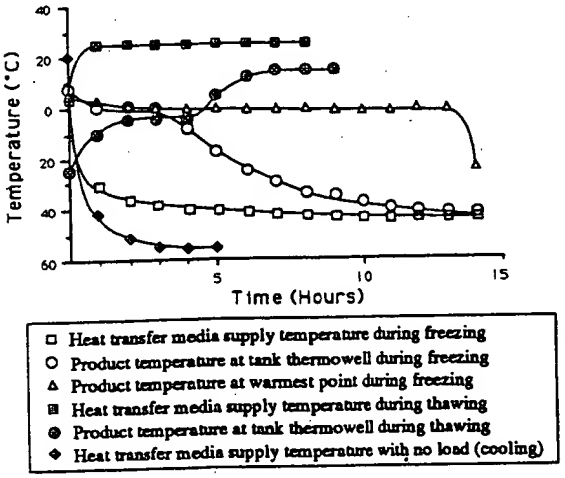

Figure 3. Freeze-thaw Temperature Profiles

Validation

Validation of the freezing endpoint can be achieved by determining where the warmest point in the vessel is located or where the final liquid cavity exists. In our case, this location has been determined to be the top center surface of the liquid. During freezing this location was the last point to achieve the desired temperature. Ideally, a temperature sensor for process control should be located at the warmest poiht in the vessel. However, to accomodate freezing of a variety of liquid volumes, the temperature sensor would need to be relocated for each volume or several temperature sensors located at strategic points would be required. For example, a thermocouple suspended on a float could place the thermocouple near the liquid surface. However, this configuration does not lend itself to aseptic design because of suspended thermocouple wires which are a hindrance to cleaning and sterilization.

The use of a thermowell located at the side of the vessel or at the bottom of the vessel was found to be an acceptable design solution. The thermowell should be located at a low level inside the tank since its location determines the minimum liquid volume allowable to be frozen or thawed with the tip of the thermowell in the solution. If temperature control is performed using a temperature sensor in a location other than the warmest point in the tank, a corresponding temperature setpoint for the thermowell must be determined based on the achievement of the desired temperature (for example -20 °C) at the warmest point in the tank. The maximum liquid volume should be used to determine the thermowell setpoint temperature. It has been proven that less than maximum volumes are thoroughly frozen upon achievement of the determined thermowell setpoint temperature.

While the freezing process continues until the temperature reaches the validated temperature setpoint, the thawing process is best terminated by a validated time setpoint. Since loose floating masses of ice exist toward the end of the thawing cycle, it is difficult to determine the thawing endpoint solely by monitoring temperature. During validation one must visually verify the end of the thawing process by verifying the absence of frozen mass. The tank must have sightglasses or ports to inspect the bulk solution for validation purposes. Also, samples f the thawed bulk solution should be taken from the top, middle and bottom of the vessel and assayed for protein concentration to verify that the thawed mass is homogeneous.

A thawing scheme has been developed based on time to thaw the maximum volume. Smaller volumes thaw within the established time to thaw the maximum volume. The temperature of the bulk solution can be controlled by stopping the supply f the warming media to the tank jacket and internal coil when a certain product temperature measured at the tank thermowell is met and restoring flow of the heating medium when the temperature of the product decreases below a certain level. During this cycling process, the liquid product continues to be recirculated causing the liquid to cool and the frozen mass to recede.

Determination of the thawing media temperature should be based on stability data of the product since a film layer of product is subject to the temperature of the warming media at the heat transfer surfaces. For the purpose of thawing at the maximum possible rate, the temperature of the media used for thawing should be the maximum temperature that the product will tolerate for a reasonable period of time. For

protein products frequently this may not be more than room temperature (about 25 °C).

	Sample Position (in tank)	pH 7.1 ± 0.1	Protein 1.5 ± 0.1 mg/ml	Activity ≥1.9 U/mg	Single Chain > 90 %
Freeze Cycle 1	Top	7.1	1.490	1.990	94.70
	Middle	7.1	1.480	1.910	94.80
	Bottom	7.1	1.440	1.930	94.10
Freeze Cycle 2	Top	7.1	1.430	2.030	94.50
	Middle	7.1	1.440	2.030	94.70
	Bottom	7.1	1.420	2.030	94.40
Freeze Cycle 3	Top	7.1	1.450	2.010	94.40
	Middle	7.2	1.430	2.020	94.50
	Bottom	7.1	1.420	2.020	94.60

Figure 4. Effect of repeated freeze-thaw cycles on a protein product (example results)

Figure 4 shows the effects of repeated freezing and thawing of a protein product in a 150 liter stainless steel portable tank. The product was frozen to -20 °C and subsequently thawed with 25 °C warming media for 9 hours. The data illustrates the ability to freeze-thaw multiple times without deleterious effects. The top row indicates the desired product specifications. Product has been stored at -20 °C for over two years without degradation.

Conclusion

Before proceeding to freeze and thaw large volumes of protein solutions the stability of proteins at various temperatures should be known. The eutectic temperature should be known as well as the stability at room temperature. The behavior of the formulating buffers during cooling, freezing, and concentration should be investigated. If bulk freezing is considered part of the process from the outset, the formulation buffer should be developed to provide eutectic freezing and protein stability at warmer temperatures, for example, at -20 °C rather than at -70°C.

The design of the freeze-thaw container should include extended heat transfer surfaces and provide division of the liquid volume into compartments to prevent cryoconcentration effects. In the case of aqueous solutions, precautions should be taken to minimize structural stress in the freezing containers caused by product expansion. Heat exchange surfaces should be designed to cause freezing to proceed from the bottom to the top of the container and to avoid formation of entrapped liquid cavities which may damage the vessel upon freezing.

Forced convection by recirculation of product during thawing from the bottom of the vessel to the top and over the ice mass is an effective method for accelerating the thawing rate and maintaining homogeneity of the bulk protein solution.

If the protein product is a human therapeutic, then the container design must meet cGMP requirements. The stainless steel tank design should be configured for clean-in-place and sterilization-in-place procedures and be capable of holding sterile product.

Scaling up of the process of freezing and thawing from vials to the large scale should be approached with caution due to uncertainty of cryoconcentration effects. If freezing and thawing of the protein product can be successfully achieved in vials it is possible that freeze-thaw can be accomplished on the large-scale.

Full testing of the product for stability after freezing and thawing for multiple cycles should be performed to examine the detrimental effects of freezing and thawing including assays on the protein structure, protein concentration, protein activity, pH, color and appearance, and specific binding assays.

Generally the warmer the allowable storage temperature the more economical and practical is the application for large-scale freezing and thawing. For freezing to temperatures less than -50 °C, mechanical refrigeration methods may not be practical. Also, freezer storage and frozen bulk handling becomes a problem with larger bulk size and lower storage temperatures.

The mechanical-based refrigeration system is a feasible design for the freezing and thawing of bulk protein solutions. Careful attention should be paid to its overall design and component selection.

Acknowledgements

The authors wish to thank Steve Phillips for his support in the early stages of the project, and to Robert Baffi for the analytical support.

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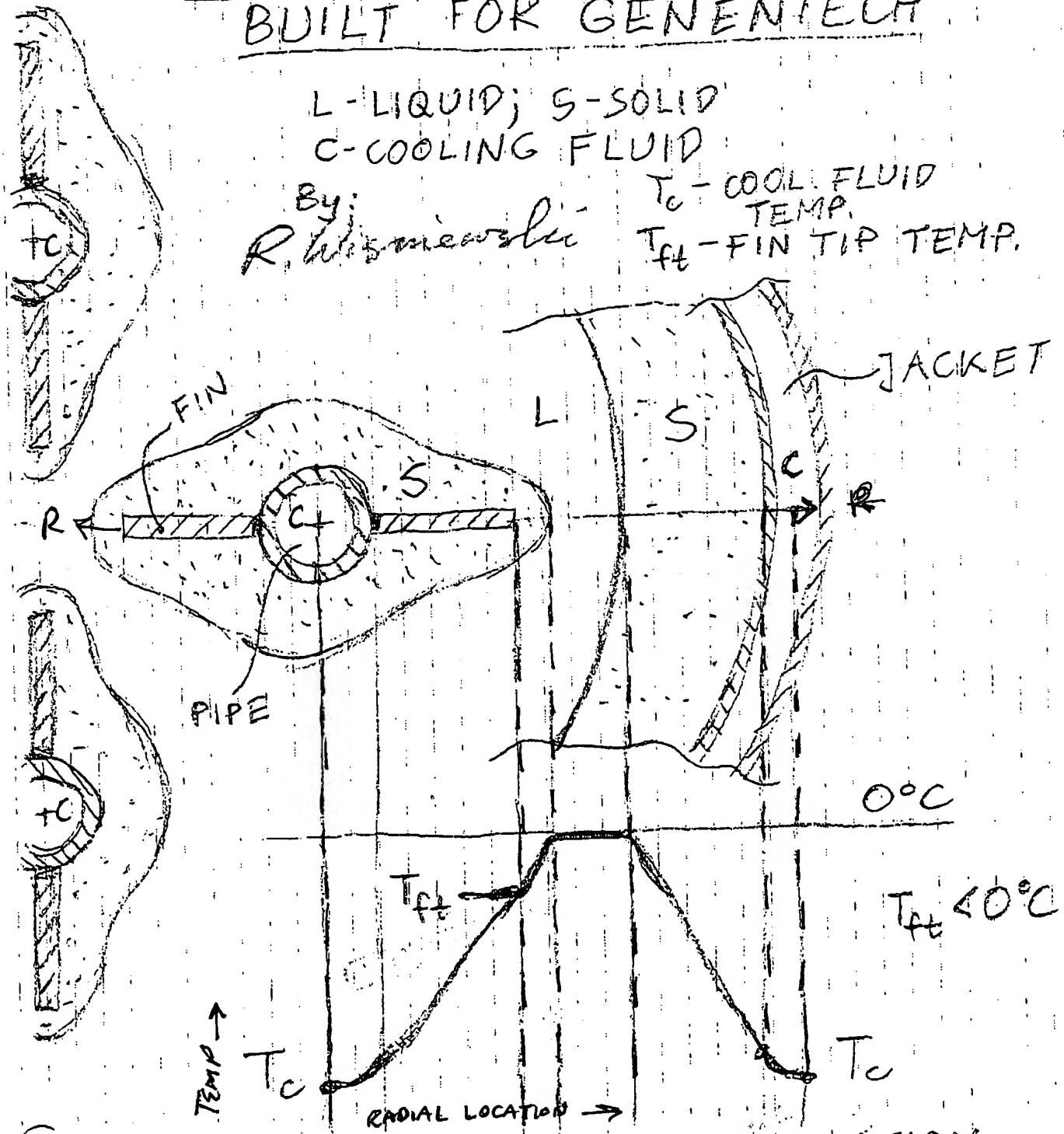
TO: NICK MESITI
JAN. 05, 2002 (1.)

FREEZING IN 103 L VESSEL BUILT FOR GENENTECH

L - LIQUID; S - SOLID
C - COOLING FLUID

By: R. Wisniewski

T_c - COOL. FLUID TEMP.
T_{ft} - FIN TIP TEMP.



TEMPERATURE DISTRIBUTION

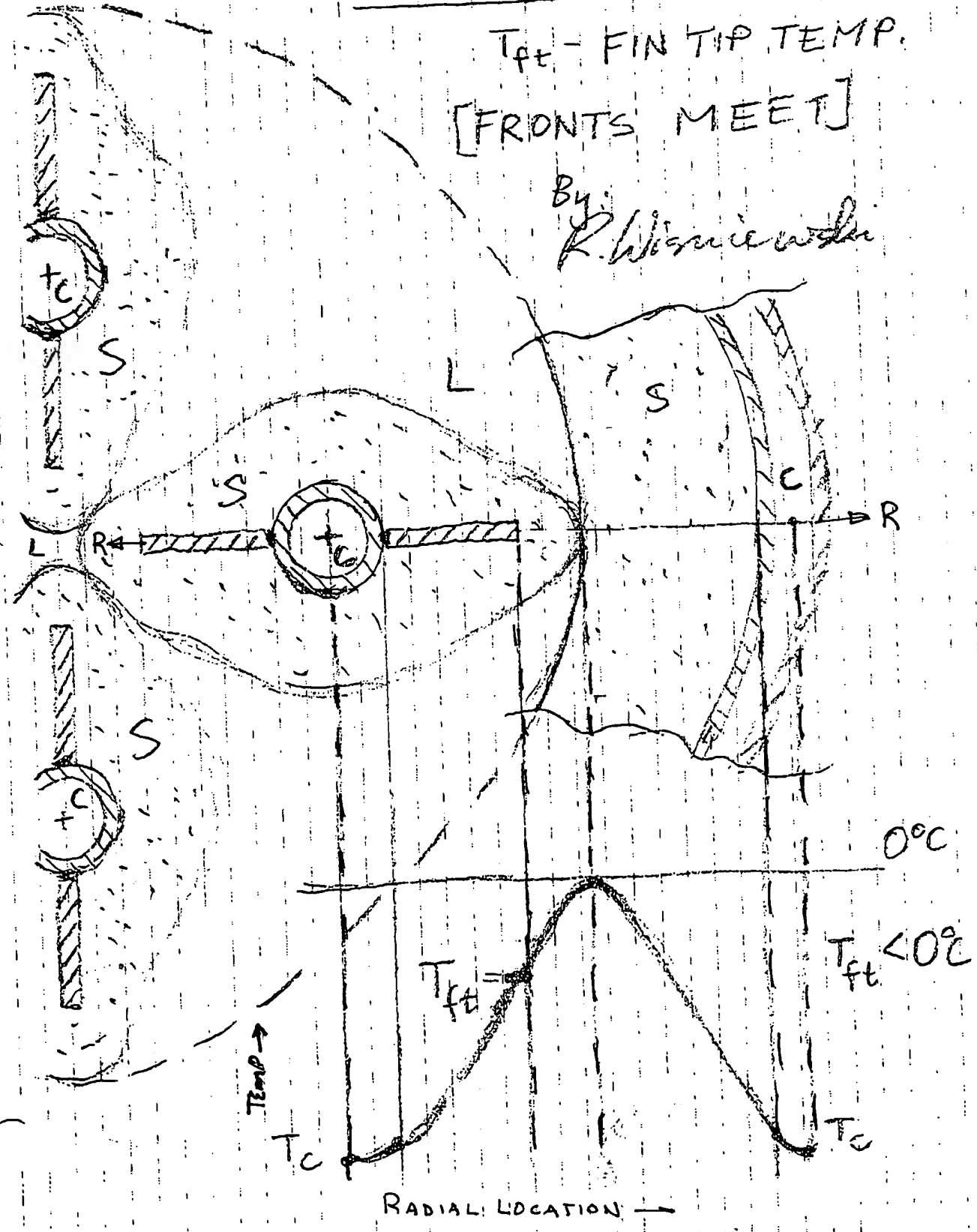
JAN. 05. 2002 (2)

103L GENENTECH VESSEL

T_{ft} - FIN TIP TEMP.

[FRONTS MEET]

By: R. Wisniewski



JAN. 5, 2002 (3.)

103L GENENTECH VESSEL

LIQUID CAVITIES CLOSING

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